Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

CONCERNING THE PRODUCTION OF CYTOTOXIC SERA BY THE INJECTION OF NUCLEOPROTEIDS.*†

RICHARD M. PEARCE, M.D.,
Professor of Pathology and Bacteriology,
AND
HOLMES C. JACKSON, PH.D.,
Adjunct Professor of Physiological Chemistry and Experimental Physiology, Albany Medical College.
(*From the Bender Laboratory, Albany, N. Y.*)

THIS investigation was incited by the publication of Beebe's studies[1] which describe the production of specific cytotoxic sera for the liver, kidney and pancreas as the result of treating animals with the respective nucleoproteids of these organs. As one of us[2] had previously made a somewhat extensive study of cytotoxic sera prepared by the injection of various somatic cells, and had reached conclusions which did not justify the views then current concerning the specificity of these bodies, it seemed advisable, in view of Beebe's very definite results, to further pursue these studies.

THE DEVELOPMENT OF OUR KNOWLEDGE OF THE CYTOTOXINS.

It may not be out of place to interpolate here a few words concerning the very definite change in opinion which has occurred during the past few years concerning the specificity of the cytotoxins. Shortly after the early publications of Ehrlich and his associates concerning the production of immune hemolysin, a vast number of publications describing specific sera for practically all cells of the body appeared in rapid succession. In most of these studies an attempt was made to demonstrate a specificity according to morphological rather than receptorial affinity in disregard of the very clear and definite statement of Ehrlich and Morgenroth. Those who denied a morphological specificity based their opinion on the observation that by the injection of cells, as those of the testicle, apparently free from blood, there resulted not only an antiserum for these cells, but also an hemolysin. This was explained by those who believed in morphological specificity by assuming that a number of red blood corpuscles, sufficient to produce a hemolysin, had been injected with the semen. Later, however, the production by a large number of investigators of hemolysin by the injection of fluids entirely free from red corpuscles, as serum and urine, demonstrated the production of antibodies from free receptors and firmly established Morgenroth's contention that specificity is a matter, not of cells, but of receptors. Despite these very conclusive demonstrations later investigators, who attempted to pro-

---

* Received for publication September 13, 1906.

† Conducted under a grant from the Rockefeller Institute for Medical Research. Read before the Section in Pathology of the British Medical Association, Toronto, August 21, 1906.

duce antisera for the cells of various organs, continued to use emulsions of unwashed organs in utter disregard of the presence of free receptors in the organ juices and also without consideration of the antibodies certain to be produced by the red cells normally present. That sera so produced possessed very definite hemolytic and hemagglutinative properties capable of producing lesions (hemoglobinuria, thrombosis, liver necroses) which might erroneously be ascribed to the supposedly specific body was first suggested by Pearce[3] in a study of nephrotoxins and later[2] definitely demonstrated by a study of the action of sera prepared from the liver, kidney, pancreas, and adrenals completely freed from blood by prolonged washing through the blood vessels. Further, the study of sera so prepared and also of sera resulting from the injection of blood serum, bile, and urine, led to the conclusion that "the cells of various organs of the body have, while differing in morphology and function, certain receptor characteristics in common, and that one type of cell may therefore produce antibodies affecting several cells of differing morphology but with like receptor groups." Specificity in the morphological sense could not be demonstrated.

Other investigations with sera prepared from washed organs, especially those of Portis[4] (antithyroid serum) and Woltmann[5] also indicate the non-specificity of the cytotoxins.

Thus, while the study of the cytotoxins has aided and may still aid in the development of our knowledge of immunity and has incidentally offered several valuable methods of experimentally reproducing pathological conditions, it is evident that the sera produced by the injection of mixtures of organ cells have not a degree of specificity sufficient to warrant their further study in the hope of developing therapeutic methods.

IMMUNIZATION TO NUCLEOPROTEIDS.

In order to prevent the adventitious formation of those bodies resulting from impure methods of immunization and also in the hope of obtaining greater specificity, a few investigators have utilized the proteid constituents of the cell.

Such attempts to produce antisera for somatic cells are limited, so far as we are aware, to those made by Marrassini,[6] Bierry[7] and his associates, and by Beebe. Of these studies the most comprehensive and most recent is that of Beebe.

Marrassini, working with the rabbit and guinea-pig, studied the effect of serum prepared by injecting rabbit's liver nucleoproteid. The changes resulting were, in general, degenerative in nature and similar to those caused by direct injection of the nucleoproteid itself, but usually more severe. The serum affected not only the organ for which it would be assumed it had an affinity, but also, to a greater extent, the kidney. Marrassini concludes that the action of a cytotoxin is of the same nature as that of other poisons of general action, such as potassium chromate, vinylamin, and pyrogallic acid, which are predisposed to affect more especially the excretory organs.

The experiments of Bierry and his associates are few in number and reported but meagerly. In the first communication Bierry describes the action of a serum prepared by injecting rabbits with the nucleoproteids of the dog's kidney. The injection of 20 to 24 c.c. of the serum into the peritoneal cavity of a dog weighing 12 to 15 kilos caused, after three or four days, an albuminuria which reached its maximum on the 10th to the 15th day. The number of experiments is not stated, but mention is made of one dog in which an albuminuria persisted for one and a half months.

Later in association with Pettit, Bierry made a comparison between the action of liver and kidney nucleoproteid sera. Each was found to cause albuminuria and degenerative changes in both liver and kidney, but the kidney serum produced a more severe albuminuria and more marked histological changes in the kidney than did the liver serum. The lesions of the kidney are described as interstitial hemorrhage, congestion of the glomeruli, and granular degeneration of the cells of the tubules with granular tube casts; and those of the liver as congestion and granular and vacuolar degeneration. The authors concluded that the action of such sera is more energetic upon the organ from which the nucleoproteid is derived, but that there is also a definite action on other organs.

In a third investigation Bierry, assisted by Mayer, made a special study of the urine of dogs receiving a liver nucleoproteid serum. They found biliary pigments, lactic acid, and homogentisic acid and demonstrated that, in some instances, the urine had a marked reducing power not due to the presence of dextrose. By various experiments they were able to produce a condition which they considered analogous to alimentary glycosuria and due to the changes produced in the liver by the serum. The histological changes in the liver were those of granular degeneration and fatty transformation. The kidney and pancreas were normal. Albuminuria occurred in two animals, but was slight and transitory. These results appear to contradict those given by Bierry and Pettit in the earlier communication.

Beebe prepared a serum by injecting thymus nucleohiston of the calf which gave precipitin tests for both nucleohiston and nucleoproteid of the thymus. The more important sera were those prepared by treating rabbits with the nucleoproteids of the liver, kidney, and pancreas of the dog. These all gave definite precipitin tests with their respective nucleoproteids and caused agglutination of cell mixtures, but had no lytic action upon the same. They were all markedly hemolytic and hemagglutinative *in vitro* in dilution up to one to five, but did not cause hemoglobinuria when injected into animals.

In testing their cytotoxic action *in vivo* intraperitoneal injections of 2 c.c. per kilo of body weight were usually employed. The kidney serum caused albuminuria and acute degeneration of the kidney without changes in other organs. Albuminuria appeared generally on the fourth or fifth day, increased in amount rapidly and was accompanied by the excretion of abundant hyaline and granular casts.

The liver serum caused granular and fatty changes and focal necroses of the liver with no changes in other organs or in the urine.

The urine of animals receiving pancreas serum caused what Beebe considers a partial reduction of Fehling's solution—"the formation of an abundant green precipitate, but not the typical red deposit of cuprous oxide." Albuminuria did not occur. Histological examination of the liver, kidney, and spleen revealed no changes; a study of the pancreas was impossible on account of imperfect fixation.

The results of these experiments (five with nephrotoxin, three with hepatotoxin and two with pancreas antiserum) provide, Beebe states, "strong indications that cytotoxic sera having a high degree of specificity can be prepared from the nucleoproteids of tissues," and that it is possible "to make a serum which will act primarily on one organ." Further comment on the work of Beebe will be made in the discussion of our own results.

METHODS.

As the amount of detail involved in work of this character is very great, it was considered advisable to limit our investigation to the study of two sera rather than to a superficial study of several. As physiological disturbances are the most satisfactory indications of the injurious action of a serum, antisera were prepared for the kidney and pancreas. The experience of practically all investigators that nephrotoxin is more nearly specific than other sera and the statements of Bierry and Mayer and that of Beebe concerning reducing substances in the urine also influenced this decision.

Rabbits, in groups of three, received in the peritoneal cavity five injections of the nucleoproteids of the dog's kidney and pancreas at intervals of five to seven days; after an interval of 10 days the animals were bled. The serum thus procured was injected intravenously into dogs which had been under observation for considerable periods of time and the urine of which had been carefully examined daily.

As a control of the supposedly specific nucleoproteid sera, in order to determine if nucleoproteid serum may not have a general toxic action on animals other than that from which the nucleoproteid was prepared, rabbits were injected with the nucleoproteid* of the pancreas of the cow and the effect of this serum was tried on the dog.

As a further control, and in order to determine also whether or not antisera for nucleic acids could be obtained, rabbits were injected with the nucleic acids of the dog's pancreas and kidney.

The preparation of the nucleoproteids.—In the preparation of the nucleoproteids we have employed one of the methods recommended by Levene. This seemed particularly advantageous for two reasons. In the first place the heating of the glands to the boiling-point effectually eliminates all possibility of future autolysis, while, on the other hand, the increased ease with which the solutions filter decreases the time of preparation. We are fully aware that the various nucleoproteids respond with different degrees of ease to extraction, but the point we had in view was not to obtain the maximum yield of material, but to endeavor to produce in the shortest time the purest product possible.

Emphasis must be laid upon the fact that only blood-free organs were employed. In this direction extreme care was exercised since the exclusion of all proteids of blood origin must be of prime importance where specific gland products stand in question. By inserting a cannula into the aorta below the renal arteries and laying a ligature above the coeliac axis, an irrigation of $\frac{n}{7}$ NaCl solution was established by means of which the blood was thoroughly removed from the organs. The blood-free organs immediately removed from the animal and cut into fine pieces were pressed with a mortar and pestle through a fine-mesh sieve and the finely comminuted material mixed with from two to four times its weight of water. The gland in suspension was then brought to the boiling-point in an Erlenmeyer flask of appropriate size. After allowing the mixture to cool it was quickly filtered through a double-folded filter. The first few cubic centimeters of filtrate, invariably turbid, were thrown on the filter again until a clear fluid resulted. The product of from one to two hours' filtration might be said roughly to correspond to the amount employed in each preparation.

---

* For this material we wish to thank Professor J. A. Mandel, of the University and Bellevue Hospital Medical School.

To this was then added acetic acid until complete precipitation resulted, the amount necessary varying according to the kind of nucleoproteid. Under ordinary circumstances the nucleoproteid separated out as a fine white flocculent precipitate; only once did it become necessary to add alcohol to bring about the precipitation of this character. This precipitate was washed with distilled water by sedimentation until the washings did not respond to the biuret reaction. This procedure in the absence of a centrifuge such as that described by Beebe was accomplished in four to five hours. In order to hasten the process, siphonage of the supernatant fluid took place before complete sedimentation had been accomplished even at the risk of loss of material. The material was then suspended in water and put into solution with the smallest possible amount of sodium carbonate; the resultant solution when filtered gave a clear filtrate which was reprecipitated with acetic acid, washed several times by decantation, and dehydrated with alcohol and ether. The products were white powders. That derived from the pancreas contained 1.74 per cent P; from the kidney 2.10 per cent P.

Just previous to each injection the required amount of the powder was weighed out and dissolved in an appropriate amount of sterilized 0.5 per cent sodium carbonate into which solvent it passed readily.

It seemed to us distinctly disadvantageous that the substance should be kept in solution for any length of time even by employing cold or antiseptics as precautions against autolysis or bacterial action. That changes of a certain character do take place under such circumstances is evidenced by the fact that Beebe noticed that a fine deposit settled out in his solutions after some weeks. In only one instance in our investigation was the injection made later than a few hours after the preparation of the solution. It would be hard to give absolute figures of the actual time consumed in the preparation of the various products. Excluding the period of cooling over night, when the gland stood in a condition free from the possibility of autolysis, the procedure occupied 15 hours most of which time, however, the material was suspended in water. This period, especially in view of the fact of the initial stoppage of autolysis, would not allow sufficient time for change of the character so guarded against by Beebe. In our opinion this precaution is undoubtedly necessary, but not of the extreme importance assumed by him. The freedom of the products from blood or protoplasmic proteid impurities would seem to be the goal for which to aim.

If, with Kossel, we assume that the present nucleoproteids or nucleins are in part laboratory products not originally present in the cell but formed in the process of the separation, the proteid moiety of the compound must be derived from protoplasmic substance as distinct from the chromatin, but not necessarily different from the cell proteids (globulins or albumins). If such is the case the question as to the specificity of nucleoproteids as such must be ruled out and the proper direction to train investigation would be to attempt to prepare antibodies from various cell-proteids or nucleic acids prepared as pure as possible. The former point we still have in mind. As Beebe states, from the analysis of his preparations it is evident that the products employed by him are not true nucleoproteids but nucleins, and the molecule of the so-called protein nucleate possesses relatively less of the proteid and more of the nucleic acid radical. He also mentions that the color proteid reactions were given in only a feeble way. Certainly it would seem that the results obtained by the use of such products should be ascribed more probably to the nucleic acid than to the proteid radicals.

The products prepared by us contained less phosphorus than his, pointing to a higher proteid content, and gave characteristic results when tested with Millons, biuret, Adamkiewicz, and Molisch reactions. These reactions could not be ascribable to cell-globulins, which were removed in the process of preparation.

It seems rather dangerous to draw conclusions as to the intravenous clotting obtained when products prepared from organs containing blood are injected in the blood stream. The possibility is always at hand of the presence of fibrin ferment, thrombin, nucleohiston, or whatever may be the agent active in setting about the changes which result in the formation of fibrin in the blood. It is, we think, at present questionable whether it has yet been definitely shown that nucleoproteid, absolutely free from contamination of blood in the general sense, will cause intravenous clotting.

The preparation of the nucleic acids.—The method employed for the preparation of the nucleic acids from the pancreas and the kidney of the dog was essentially that of Levene. The finely comminuted gland was boiled for two hours in a 5 per cent solution of sodium chloride after which sodium acetate in the proportion of 7 per cent of the weight of the fresh glands was added and the mixture allowed to cool. Sodium hydroxide was next added in excess and the material allowed to stand over night. After the reduction of the greater part of the alkalinity with acetic acid the faintly alkaline mixture was precipitated with an excess of picric acid and acetic acid carefully added until a well-formed flocculent precipitate settled out. This filtered quickly. The amount of acetic acid necessary at this point varies according to the kind of nucleic acid. The proteid-free filtrate was next thrown in a large volume of 95 per cent alcohol, and allowed to stand until the nucleic acid settled out. The major part of the alcohol could be removed by siphonage and the precipitate collected on paper. The precipitate was again dissolved in alkali in the presence of sodium acetate and acidulated with acetic acid. A slight precipitate was filtered off. To the filtrate saturated with sodium chloride was added hydrochloric acid until the mixture reacted acid to congo red when an equal volume of alcohol was added. This precipitate showed itself to be biuret free and was dehydrated with alcohol and ether. The products contained from 8 to 9 per cent phosphorus. As was the case with the nucleoproteids, the necessary amount of the substance was dissolved in an appropriate amount of 0.5 per cent sodium carbonate and injected within a few hours after preparation.

DETAIL OF EXPERIMENTS AND RESULTS.

In all instances the dogs received in a small branch of the femoral vein a uniform dose of 2 c.c. of serum per kilo of body weight. The injections were made under light ether anesthesia and this experiment terminated by bleeding from the femoral artery under light chloroform anesthesia. In each group animals were killed at intervals of four, eight, and ten days. Five animals received dog's kidney nucleoproteid serum;* five, dog's pancreas nucleoproteid serum; four, cow's pancreas nucleoproteid serum; three, dog's kidney nucleic acid serum; and three, dog's pancreas nucleic acid serum.

---

* In preparing the various sera, the average amount of dog's kidney nucleoproteid to each injection was 0.11 gram; of the dog's pancreas nucleoproteid, 0.13 gram; of the cow's pancreas nucleoproteid, 0.15 gram; of the dog's kidney nucleic acid, 0.25 gram; and of the dog's pancreas nucleic acid, 0.11 gram

Histology.—In no instance did postmortem examination reveal definite macroscopic lesions. The histological study was limited to the kidney, pancreas, and liver. Tissues were fixed in formalin and in Zenker's fluid; the former were examined for fat by the frozen section and Scharlach R method, the latter were embedded in celloidin and stained by hematoxylin and eosin. Fatty changes were not constant, and, when present, of but slight degree. In the kidney nucleoproteid series, fatty changes were found in the kidney series once (10th day) and in no other organ. In the dog's pancreas nucleoproteid series very slight fatty changes were found in three—in one, in liver, kidney, and pancreas; in one, in liver and kidney; in the third, in kidney only. The cow's pancreas series gave negative results by this method. On the other hand, in the nucleic acid series fatty changes in the kidney were constant and well marked, affecting uniformly the loops of Henle. All the other organs were negative except for slight changes in the liver in one animal of the kidney nucleic acid series.

In the study of the Zenker hardened material, in no instance were changes in the pancreas found. The liver, although devoid of well-marked lesions, such as necrosis, almost constantly exhibited a peculiar change affecting all parts of the organ. This condition, which has been noted frequently by one of us as the result of the injection of various cytotoxins, and which unquestionably is that described by other investigators as a granular, vacuolar, or fatty degeneration is characterized by a swollen, pale, "washed-out" appearance of the liver cells with a few large, coarse granules and large irregular pale areas which stain but faintly or not at all. The latter areas are not of the character of fat vacuoles, but more irregular, vary considerably in size, and have no definite relation to the nucleus. The appearance is similar to that of liver cells containing glycogen, and as the same condition in marked degree was found in the adrenal, we are inclined to consider it as an abnormal accumulation of glycogen. Unfortunately this explanation did not occur to us until near the close of our experiments and proper routine tests were not made. Upon the basis of a single attempt to isolate the glycogen from the last dog of the kidney nucleic acid series, it can be stated, however, that a greatly increased amount of glycogen was present. This was determined by extracting the finely divided gland with hot water and filtering. The filtrate appeared milky white, gave the iodine reaction for the glycogen, which could be precipitated with alcohol at 65 per cent. No particular haste was employed in getting the organ into boiling water and the fact that the glycogen remained so long postmortem points either to a large excess of the compound or perhaps to the absence or great diminution during life of the enzyme in the liver which transforms the glycogen.

The changes in the kidney were purely degenerative in character, of slight degree, and almost constantly present. The cells of the tubules, especially of the convoluted tubules, were swollen and very granular with more or less definite reticulum. As a rule the nucleus could be readily seen, but in the more marked conditions it stained poorly. Rarely the entire lining of the tubule could be seen separated from the basement membranes and lying free in the lumen as a poorly staining granular ring. In addition to these changes the tubules of the cortex, and less frequently, the straight tubules contained the finely granular reticulum so commonly associated with the milder disturbances of the kidney. In the straight tubules this material was often so compressed as to suggest finely granular casts, but definite casts were found but twice and only in the kidney nucleoproteid series. In the collecting tubules and especially in the loops of Henle, in some instances, a more or less marked vacuolization was present corresponding to the fatty change determined by the use of Scharlach R In no kidney were glomerular changes found.

It is difficult to distinguish sharply between the types of kidney lesions caused by the different sera. In general the changes due to nucleoproteid sera were more severe and widespread than those due to the nucleic acid sera. The latter were characterized by a greater degree of fatty change and by a tendency to involve the loops of Henle rather than the convoluted tubules. If the presence of casts be taken as a criterion the lesions due to the dog's kidney nucleoproteid must be judged the most severe. It is a matter of considerable importance that the cow pancreas serum, in no sense specific, caused lesions in the kidney not to be distinguished from those due to other sera.

Examination of the urine.—The animals, fed on dog biscuit, were kept in cages suitable for the collection of their urine. This was examined daily for proteids and reducing carbohydrates. The clear filtrated urine was heated to boiling and the merest trace of acetic acid added. This indicated in a roughly quantitative way the amount of coaguable proteids (albumin and globulin) present. It is almost a constant occurrence to find traces of such appearing at variable and irregular times in dog's urine. By traces we mean that upon the addition of acetic acid a faint turbidity appears, which is in no sense a precipitate, but which in the course of 24 hours settles out at the bottom of the tube as a small light fluffy cloud. A second portion was tested with potassium ferrocyanide and acetic acid. The clear urine acidulated with the acid was treated with one drop of the dilute reagent. It was the unusual occurrence to find this test absolutely negative in the dogs under examination in this series of experiments and this result has been confirmed in the examination of many of the urines of other animals at different times. The urines of dogs, concentrated as it is in the passage of an excessively long system of tubules, carries down with it from the collecting tubules not inconsiderable quantities of proteids of the mucin and nucleo-albumin type, which show themselves by the latter test. At times the result of this reaction amounted to a definite flocculent precipitate. With the possible exception of the urines of two of our dogs injected with kidney nucleoproteid antiserum we feel assured that at no time did our tests give evidence of an excretion of proteid which could in any sense quantitatively or qualitatively be ascribed to changes in the kidney.

In the case of Dog 14 injected on May 20 the urine collected on the 24th showed with heat and with potassium ferrocyanide a decided flocculent precipitate which would be called a positive reaction. The same is true for Dog 15 injected on the same day, the urine reacting positively to both tests on the 25th. Other than these, no results were obtained which would compare in any way to that described by Beebe, where 53 per cent of the urinary nitrogen appeared in the form of albumin. The quantity of proteid in the cases cited was so small that no attempt was made to determine whether it was an albumin or globulin. At no time were casts obtained in the urine, but upon histological examination of the kidney of Dog 15 as in one other dog (17) of this series, a few hyaline casts were seen in the tubules.

In a paper read this year before the American Medical Association by Robertson and Hacker, and based upon work done in this Laboratory, attention was called to the frequent appearance in human urines tested by Fehling test solutions, of a yellowish-green coloration which varies in intensity from the merest opalescence to a definite, finely divided precipitate which in the course of some hours settles out slowly to the bottom of the tube. It was then stated that this reaction is the result of the addition of too much urine in performing the test and must undoubtedly be ascribed to the presence in the urine at times of some weakly reducing compounds capable in large quantities of partially reducing copper solutions. This pseudo-reaction never occurs when there is employed a small amount of urine, sufficient, however, to show dextrose if present. In testing the urine of dogs these remarks are extremely to the point. When 10 or more drops of the filtered dog's urine were added to the two or three times diluted and boiling Fehling's solution and the mixture boiled a few seconds, it was not at all uncommon to notice in a few moments the appearance of a greenish-yellowish haze which gradually became more opalescent and opaque and which, in extreme cases, might resolve itself into a greenish or yellowish precipitate settling to the bottom of the tube, sometimes immediately, or, more frequently, if left standing, over night. This reduction is not due to dextrose; the urine showed no difference before or after fermentation, polariscopically; no osazone could be obtained, and Nylander's test resulted negatively. If on the other hand, only two or three drops of the urine were added to the diluted Fehling's solution in the ordinary way and not boiled after the addition of the urine the test remained negative. All of the urines tested for dextrose in the latter way, during the course of our experiments, showed absolutely negative results, while the pseudo-reaction was obtained more or less irregularly in 16 of the 20 dogs studied. In our opinion this pseudo-reaction has no direct bearing on the subject under discussion.

In the case of one dog a continued pseudo-reaction was obtained which could be explained by the elimination of lactose following the removal of puppies during lactation. The negative results of Fehling's test with the urine of this animal indicated beautifully the fact contended for in the paper alluded to above, that Fehling's test performed as there advised can be employed without hesitation as a criterion for the presence of dextrose as against other reducing bodies. In none of the urines examined in these experiments was dextrose present in amounts which could be said to exceed the normal, granting that traces of reducing compounds, perhaps dextrose, do appear ordinarily.

While we cannot of course state positively, still it must be granted that it seems extremely likely that the reduction which Beebe describes as a "partial reduction of Fehling's solution" with "the formation of an abundant green precipitate" was nothing more or less than the pseudo-reduction which we have so frequently observed in the urines of normal dogs. As the manner in which his tests were performed or semi-quantitative details are not outlined, such explanations must be classed as only suggestive but not positive. It is our experience that the urines of the dogs injected with dog's pancreas nucleoproteid antiserum show no different character, as regards the content of reducing carbohydrates, than those treated with any of the other sera.

CONCLUSIONS.

In so far as conclusions from so small a number of experiments are justifiable, our results do not support the theory that specific cytotoxic sera may be developed by the injection of nucleoproteids but indicate, rather, that such sera have certain mildly toxic properties acting in a general way and affecting especially the principal excretory organ, the kidney. In this regard we are in accord with Marrassini and in a general way with Bierry and his associates, but in direct conflict with Beebe's very definite statements.

It is clearly evident, however, that Beebe's claims for a specific pancreas serum based on the demonstration of reducing substances in the urine, especially in the absense of histological changes in the pancreas, is untenable in view of our experience with like reducin substances more or less constantly present in the urine of normal dogs.

We can make no definite statement concerning nucleoproteid hepatotoxic serum, as we did not prepare such, but it is difficult to explain the focal necrotic lesions of the liver, caused by Beebe's hepatotoxin, as a result of the action of a substance which should, presumably, affect the liver cells more diffusely. It may or may not be a matter of importance that his nucleoproteids were prepared from organs but roughly washed in running water and, therefore, especially as regards the liver, containing large amounts of blood. That a very toxic nucleoproteid may be obtained from red blood cells is well known and it is possible that, as a result, Beebe's serum may have contained hemolysin and agglutinin due to this substance, in addition to the supposed hepatotoxin, in sufficient amount to cause focal liver necroses through agglutinative thrombi, as has previously been described by one of us.[8]

Beebe states that all his sera "are markedly hemagglutinative and hemolytic *in vitro* in dilution up to one to five, but in dilution of one to twenty or above no difference is to be detected between the immune and the normal serum." The hemolytic and hemagglutinative powers of our nucleoproteid sera (the nucleic acid sera were not tested) were much weaker. Thus the dog's pancreas nucleoproteid serum, for example, had very slight power to hemolyze or agglutinate red cells. Two parts of the pure serum to one dog's blood caused neither hemolysis nor agglutination, while 10 parts were required to cause but a faint hemolysis; with the same amount a very definite fine agglutination was evident only after five minutes. It would appear, therefore, by comparison, that the use of blood-free organs in the preparation of nucleoproteid sera lead to the production of a serum less hemotoxic than those developed by the use of unwashed organs. Under such circumstances, therefore, it is very important that future investigators of nucleoproteid sera work with blood-free tissues.

The changes in the kidney resulting from the administration of the kidney nucleoproteid serum, while very definite, cannot be considered as specific or even "special," for similar changes, often of the same grade, characterized the action of all other sera, even that prepared from cow's pancreas. These changes are, therefore, to be regarded as the effect of the excretion of toxic substances rather than as the manifestation of a selective action of the serum. It must be admitted, however, that it is difficult so to explain the severe albuminuria described by Beebe.

Finally, attention must once more be called to the importance, in all investigations of cytotoxic sera, of the use of blood-free organs, to the occasional occurrence of copper-reducing compounds in the urine of apparently normal dogs, to the not infrequent spontaneous nephritis in these animals, and to the possibility of albuminuria due to a catheter cystitis.

REFERENCES.

1. BEEBE, S. P. Cytotoxic Serum Produced by the Injection of Nucleoproteids, *Jour. Exper. Med.*, 1905, 7, p. 733.
2. PEARCE, R. M. Concerning the Specificity of the Somatogenic Cytotoxins, *Jour. Med. Res.*, 1904, 12, p. 1.
3. ——— An Experimental Study of Nephrotoxins, *Univ. Penn. Med. Bull.*, 1903, 16, p. 217.
4. PORTIS, N. M. Experimental Study of Thyrotoxic Serum, *Jour. Infect. Dis.*, 1904, 1, p. 127.
5. WOLTMANN, H. A Study of the Changes in the Blood and Blood-Forming Organs Produced by Cytotoxic Sera, *Jour. Exper. Med.*, 1905, 7, p. 119.
6. MARRASSINI, A. Richerche sopra l' azione tossica dei nucleoproteidi de fegato, *La clinica moderna*, 1903, No. 13. Reference in *Biochem. Centralbl.*, 1903, 1, p. 805.
7. BIERRY, H. Recherches sur les néphrotoxines, *Compt. rend. de la Soc. de Biol.*, 1903, 55, p. 476.

   BIERRY, H., and PETTIT, A. Sur le pouvoir cytotoxique des certains sérums consécutif à l'injection de núcléoprotéides, *ibid.*, 1904, 56, p. 238.

   BIERRY, H., and MAYER, A. Sur l'action du sang rendu hépatotoxique par injections intrapéritonéales de nucléoprotéides du foie, *ibid.*, p. 1016.
8. PEARCE, R. M. The Experimental Production of Liver Necroses by the Intravenous Injection of Hemagglutinins, *Jour. Med. Res.*, 1903, 12, p. 329; *ibid.*, 1906, 14, p. 541; Experimental Cirrhosis of the Liver, *Jour. Exp. Med.*, 1906, 8, p. 64.